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Purification of Intensive Shrimp Farming Effluent by *Gracilaria* Coupled with Oysters

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Abstract: In this study, we explored the ability of *Gracilaria vermiculophylla* coupled with *Crassostrea hongkongensis* to purify aquaculture effluent by analysing the purification of intensive shrimp farming effluent using *G. vermiculophylla* under different environmental conditions. After determining the optimal conditions, we further investigated the capability of the *G. vermiculophylla* and oyster coupling in intensive shrimp farming effluent purification. The shrimp farming density was 200 individuals per cubic metre (equivalent to 0.2 individuals per litre). The optimal environmental parameters were as follows: oyster biomass of 4.5 kg·m^{-3}, *G. vermiculophylla* biomass of 2 kg·m^{-3}, water temperature of 25–30 °C, and salinity of 15–30‰; the total inorganic nitrogen, PO_4^{3-}-P, total nitrogen, and total phosphorus removal rates were 59.56%, 97.43%, 63.67%, and 76.25%, respectively, with *G. vermiculophylla* increasing in weight by 31.01%. For every 1 kg increase in the dry weight of *G. vermiculophylla*, 36.89 g of N and 12.40 g of P could be absorbed from the effluent. Our findings indicate that the coupling of *G. vermiculophylla* with oysters greatly contributed to the purification of effluent from intensive shrimp farming and can, thus, be used for treating intensive shrimp farming effluent.

Keywords: sustainability; purification; *macroalgae*; *Crassostrea hongkongensis*

Key Contribution: The coupling of *Gracilaria* and oysters significantly decreased the levels of N and P in effluent from intensive shrimp farming. This aquaculture effluent purification method not only improves water quality and the surrounding environment, but also provides new ideas for the recycling of aquaculture effluent.

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1. Introduction

Intensive aquaculture is a high-density, high-efficiency farming method that plays an important role in increasing the yield and quality of farmed animals and ensuring the supply of aquatic products to the market [1]. Recently, biofloc-mediated intensive aquaculture has become a popular, eco-friendly farming model [2] that not only achieves high production, but also improves the physiological health of farmed animals, reduces

water exchange, and increases feed utilisation; thus, it has economic and ecological significance [3,4]. The biofloc farming model can effectively control harmful substances, such as ammonia nitrogen (NH_4^+-N) and nitrite (NO_2^--N), in culture water within a safe concentration, facilitating a closed and efficient culture system. However, large amounts of nitrate (NO_3^--N) and phosphate (PO_4^{3-}-P) accumulate in the water in the later culture stages for this technique [5]. If discharged directly, high concentrations of N and P can cause eutrophication in the receiving water body [6], polluting the surrounding ecological environment. Therefore, effectively treating N and P in intensive shrimp farming effluent and recycling its resources are the main approaches to effluent purification. Biological flocculation systems are an emerging technology for effluent treatment that can effectively remove pollutants, such as N and P, from wastewater by interacting with microorganisms, suspended solids, and dissolved organic matter [7]. They also enhance the immunity and growth of cultured organisms. In biological flocculation systems, it is often necessary to combine other treatment methods to further improve the effectiveness of the effluent treatment. Common aquaculture effluent treatment methods include physical sedimentation, chemical coagulation, and biological filtration [8–10]. Physical sedimentation uses gravity to settle suspended particles, but has limited effectiveness in removing dissolved pollutants. Chemical coagulation uses coagulants to form flocs that precipitate pollutants, but may introduce secondary pollution. Biological filtration removes pollutants through microbial metabolism and has good treatment effects; however, its efficiency may be insufficient when treating high-concentration effluents. At present, low-cost and highly beneficial biological purification methods are research hotspots for intensive shrimp farming treatment effluent [11]. The use of macroalgae coupled with filter-feeding shellfish to treat effluents from intensive shrimp farming is a typical biological treatment method [12].

Gracilaria and oysters not only are of high economic value, but also can be used for purifying aquatic environments. They have fast growth, high nutritional value, and strong environmental adaptability [13]. Macroalgae play an important role in water purification. They grow and reproduce by absorbing and utilising nutrients in the water, thereby decreasing the amounts of N and P [14]. *Gracilaria*, a large red alga widely distributed in intertidal zones along the coasts of China, has important economic and medicinal value [15]. *Gracilaria* has a good capacity for absorbing N and P nutrients and heavy metals from water, and exhibits good adaptability to eutrophic environments, making it suitable for purifying intensive shrimp farming effluent [16,17]. *Crassostrea hongkongensis* is the main shellfish species in brackish water convergence areas along the coasts of Guangxi, western Guangdong, and Fujian, China [18], and it has fast growth, high nutritional value, and good environmental adaptability [19]. Through filter-feeding and water-filtering actions, it can consume phytoplankton from the water and decrease the number of suspended particles [20]. Oysters are filter-feeding bivalves that convert nitrogen from foods, such as plankton, into biomass during cultivation. However, during metabolism, nitrogen is released by oysters into the surrounding water in the form of NH_4^+-N [21]. This may not only have a negative impact on the growth of the oysters themselves, but may also pollute the entire culture environment. As a large seaweed with high nitrogen absorption capacity, *Gracilaria* has an affinity for ammonia nitrogen, which enables it to effectively absorb ammonia nitrogen from seawater, thereby decreasing its concentration and improving water quality. This particular combination not only decreases the accumulation of ammonia nitrogen during oyster cultivation, but also provides the nutrients needed for the growth of *Gracilaria*, forming a mutually beneficial symbiotic ecological system [22]. Biofloc systems are known to produce large amounts of total suspended solids (TSS); therefore, it is reasonable to add oysters for filtration [23]. Using a combination of *Gracilaria* and oysters

in a biofloc system to treat shrimp culture effluent is expected to achieve efficient pollutant removal and resource recovery.

There have been many reports on the degradation of N and P nutrients by large seaweeds. For oysters, studies have only been conducted to analyse the effects of treatment on suspended particles in aquaculture effluent [24–26]. Systematic research on the purification effects of *Gracilaria* coupled with oysters on effluents from intensive shrimp farming has rarely been reported. In this study, different densities, salinities, and temperatures were tested, aiming to explore the impact of these environmental factors on the efficiency of the *Gracilaria* and oyster combination in treating shrimp aquaculture effluent. The density of red sea urchins has a negative impact on the density of macroalgae attached to kelp. The grazing behavior of red sea urchins significantly reduces the density of macroalgae. The density of macroalgae decreases with increasing water depth, but increases with increasing water flow velocity [27]. Salinity was studied because shrimp farming is usually conducted under different salinity conditions, and understanding the treatment efficiency of *Gracilaria* and oyster combinations under various salinities can help broaden the applicability in effluent treatment for actual farming production [28]. Temperature is a key factor affecting the growth, physiology, and niches of macroalgae. Most macroalgae can grow within the temperature range of 15–25 °C. Seasonal changes cause temperature fluctuations, which, in turn, affect the metabolic activities of macroalgae and their ability to absorb pollutants [29].

Effluent from intensive shrimp farming was used as the basis of this study to explore the adaptability and N and P absorption effects of *Gracilaria* in treating effluent from intensive shrimp farming under different environmental conditions. We analysed and determined the suitable biomass and growth environment parameters for *Gracilaria*, and then conducted coupling experiments with *C. hongkongensis* to explore their purification ability and N and P deposition effects in order to provide a basic reference for establishing a purification technology for effluent from intensive shrimp farming and achieving "resource utilisation".

2. Materials and Methods

2.1. Purification of Nitrogen and Phosphorus in Intensive Shrimp Farming Effluent by Gracilaria Under Different Environmental Conditions

2.1.1. Experimental Materials

Gracilaria

G. vermiculophylla was collected from a beach aquaculture area in Lingmen Town, Dianbai District, Maoming City, Guangdong Province, and was first cultured in five 100 L experimental tanks. The temporary culture conditions were as follows: seawater salinity, 30‰; pH, 8.2; and temperature, 26 °C. In four tanks, a certain amount of fresh water was added daily. We added 20% of the total water volume of the experiment as fresh water daily for the acclimatization and temporary culture of *Gracilaria* [30]. The temporary culture period lasted for 14 days. At the end of dilution, the salinity in each culture tank was 5, 10, 15, 20, or 30‰.

Experimental Water

Effluent was obtained from a zero-water-exchange biofloc shrimp-farming pond system at a culture farm in Maoming City, Guangdong Province. The shrimp farming density was 200 individuals per cubic metre (equivalent to 0.2 individuals per litre). The water temperature, dissolved oxygen, pH, NH_4^+-N, NO_2^--N, NO_3^--N, PO_4^{3-}-P, and TSS were 27 °C, 4.56 mg·L^{-1}, 7.6, 0.42 mg·L^{-1}, 0.65 mg·L^{-1}, 524.51 mg·L^{-1}, 50.81 mg·L^{-1}, and 335.25 mg·L^{-1}, respectively. We diluted the biofloc water with brackish water at a certain ratio (about 1:50).

2.1.2. Experimental Design

Experiment 1: *Gracilaria* Density test

The experimental system consisted of a 15 L foam box (0.5 m × 0.15 m × 0.2 m, no aeration) filled with 10 L of intensive shrimp farming effluent (salinity 15‰, pH 8.3, temperature 23.5 °C, Dissolved Oxygen (DO) 6.52 mg·L^{-1}, alkalinity 152 mg $CaCO_3$·L^{-1}). The experiment included a low-biomass group (LBG), medium-biomass group (MBG), high-biomass group (HBG), and blank control group (Ctrl). The LBG, MBG, and HBG groups corresponded to *Gracilaria* biomasses of 1, 2, and 4 kg·m^{-3}, respectively, while the Ctrl group did not contain *Gracilaria* (three replicates per group).

Experiment 2: *Gracilaria* Temperature test

The experiment was divided into four groups, namely 20, 25, 30 °C, and a blank control group (Ctrl). *Gracilaria* biomass of 2 kg·m^{-3} was added to the 20, 25, and 30 °C groups, while the Ctrl group did not contain *Gracilaria*; average temperature (26.5 ± 1.5 °C) conditions were applied. The initial water quality parameters of each group were basically stable at: salinity, 15‰; pH, 8.5; DO, 6.70 mg·L^{-1}; and alkalinity, 160 mg $CaCO_3$·L^{-1}.

Experiment 3: *Gracilaria* Salinity test

The experiment was divided into five groups: intensive shrimp farming effluents with salinities of 5, 10, 20, and 30‰, and a blank control group (Ctrl). Then, *Gracilaria* biomass of 2 kg·m^{-3} was added to each tank, while the Ctrl group did not contain *Gracilaria*. Intensive shrimp farming effluent with a salinity of 15‰ was used as the control group, and the temperature was average temperature (26.5 ± 1.5 °C). The other initial water quality parameters of each group were basically stable at: pH, 8.5; DO, 6.55 mg·L^{-1}; and alkalinity, 165 mg $CaCO_3$·L^{-1}.

In the above experiments, each group was tested with three replicates, and the water quality indicators were measured every 3 days. The wet mass of *Gracilaria* was measured at the beginning and end of the experiment. The light intensity was controlled at 1000–2000 lx with a light/dark ratio of 12:12. Each experiment lasted for 18 days.

2.1.3. Water Quality Measurement

Water samples were collected from each group on days 0, 3, 6, 9, 12, 15, and 18. According to the "Marine Survey Specifications" (GBT 12763.4—2007) [31], the NH_4^+-N, NO_2^--N, NO_3^--N, and PO_4^{3-}-P concentrations in the water samples were determined using the indophenol blue spectrophotometric, hydrochloric acid naphthylamine spectrophotometric, zinc–cadmium reduction, and phosphomolybdic blue spectrophotometric methods, respectively. The total inorganic nitrogen (TIN) was calculated as the sum of the concentrations of NH_4^+-N, NO_2^--N, and NO_3^--N.

2.2. Purification of Intensive Shrimp Farming Effluent by the Coupling of Gracilaria and Oysters

2.2.1. Experimental Materials

Oysters

C. hongkongensis was purchased from an oyster farm in Lingmen Town, Dianbai District, Maoming City, Guangdong Province, China. Oysters with an average individual weight of 120.87 ± 18.13 g were selected, and surface attachments were removed before temporarily culturing them for 7 days. The temporary culture conditions were as follows: salinity, 15‰; pH, 8.2; temperature, 25.1 °C; and dissolved oxygen, 7.10 mg·L^{-1}.

Experimental Water

The intensive shrimp farming effluent was obtained from a zero-water-exchange biofloc shrimp-farming pond system at an aquaculture farm in Maoming City, Guangdong Province. The water temperature, dissolved oxygen, pH, NH_4^+-N, NO_2^--N, NO_3^--N,

PO_4^{3-}-P, and TSS were 26 °C, 4.70 mg·L^{-1}, 7.7, 1.95 mg·L^{-1}, 2.90 mg·L^{-1}, 235.02 mg·L^{-1}, 21.41 mg·L^{-1}, and 219.60 mg·L^{-1}, respectively. We diluted the biofloc water with brackish water at a certain ratio (about 1:10).

2.2.2. Experimental Design

Prior to the experiment, oysters with high activity were randomly selected. A mixture of 740 L of brackish water and 60 L of biofloc-based intensive shrimp farming effluent was prepared as 800 L of culture water and placed in a 1000 L experimental tank. The experiment involved an experimental group (CSG) and a control group (Ctrl), each in triplicate, with each replicate in a 1000 L experimental tank. Oyster biomass of 3 kg·m^{-3} and *Gracilaria* were added to the experimental tanks under the previously determined optimal conditions. The oysters were evenly placed in a circular plastic frame (diameter, 0.5 m), which was suspended 0.3 m below the water's surface. *Gracilaria* was placed in a white mesh bag and submerged 0.6 m below the water's surface; natural light conditions of 2000–10,000 lx were used, and the water temperature was maintained at 25 °C. The control group was not added (neither oysters nor *Gracilaria* were added). The experiment lasted for 48 days, during which, nanotube aeration (Shanghai Jiarongtong Network Technology Co., Ltd., Shanghai, China) was employed to ensure sufficient dissolved oxygen.

2.2.3. Water Quality Measurement

Water samples were collected from each group on days 0, 6, 12, 18, 24, and 48, and the indicators NH_4^+-N, NO_2^--N, NO_3^--N, PO_4^{3-}-P, and TIN were measured. Total nitrogen (TN) and total phosphorus (TP) were measured using the potassium persulfate oxidation method, and TSS and chemical oxygen demand (COD) were measured using the gravimetric and alkaline permanganate methods, respectively. The TSS and alkalinity of the water body were determined by the gravimetric method and the pH method, in accordance with the Chinese national standard (GB/T 17378.4-2007) [32].

2.3. Deposition Effects of Nitrogen and Phosphorus in Effluent by Gracilaria–Oyster Coupling

2.3.1. Experimental Materials

The samples of *Gracilaria* used were from the initial and final stages of the first two studies. BG_0, T_0, S_0, and CSG_0 represent the initial *Gracilaria* density group, the initial *Gracilaria* temperature group, the initial *Gracilaria* salinity group, and the initial *Gracilaria*–oyster coupling group, respectively. LBG_{18}, MBG_{18}, and HBG_{18} represent the *Gracilaria* 1 kg·m^{-3} group, *Gracilaria* 2 kg·m^{-3} group, and *Gracilaria* 4 kg·m^{-3} group at the end of the 18-day *Gracilaria* density experiment, respectively. Furthermore, 20 $°C_{18}$, 25 $°C_{18}$, and 30 $°C_{18}$ represent the *Gracilaria* 20 °C group, *Gracilaria* 25 °C group, and *Gracilaria* 30 °C group at the end of the 18-day *Gracilaria* temperature experiment, respectively. Also, 5_{18}, 10_{18}, 20_{18}, and 30_{18} represent the *Gracilaria* salinity 5‰ group, *Gracilaria* salinity 10‰ group, *Gracilaria* salinity 20‰ group, and *Gracilaria* salinity 30‰ group at the end of the 18-day *Gracilaria* salinity experiment, respectively. CSG_{48} represents the *Gracilaria*–oyster coupling group at the end of the 48-day experiment.

2.3.2. Sample Processing and Indicator Detection

The experimental samples were processed by vacuum freeze-drying (Lab-1D-80, Shanghai Precision Instruments Co., Ltd., Shanghai, China) to directly remove water from the samples in the form of ice sublimation. The *Gracilaria* samples were pre-frozen at –80 °C and freeze-dried for 48 h before being ground, sieved, and stored dry. The TN and TP contents in the freeze-dried *Gracilaria* powder samples were determined according to the national standards GB17378.4-2007 and GB17378.5-2007 [33].

2.4. Data Analysis

The weight gain rate (WGR) of *Gracilaria* was calculated by the following formula:

$$WGR = (Wt - W0)/W0 \times 100\% \quad (1)$$

where Wt is the wet mass measured at sampling, and W0 is the initial wet mass.

The removal rate (R) for each water quality indicator was calculated using the following formula:

$$R = (C0 - Ct)/C0 \times 100\% \quad (2)$$

where Ct is the concentration at sampling, and C0 is the initial concentration.

The survival rate (SR) of oysters was calculated by the following formula:

$$SR = (Nt - N0)/N0 \times 100\% \quad (3)$$

where Nt is the number measured at sampling, and N0 is the initial number.

The content and net increase per unit dry weight of *Gracilaria* were calculated as follows:

$$\text{Content per unit dry weight} = Md0/M0 \text{ or } Mdt/Mt \quad (4)$$

$$\text{Net increase per unit dry weight} = Md0/M0 - Mdt/Mt \quad (5)$$

where M0 is the wet mass measured at the initial sampling, Mt is the wet mass measured at the end, Md0 is the freeze-dried mass at the initial sampling, and Mdt is the freeze-dried mass at the end of the experiment.

The increases in TN and TP in the *Gracilaria* freeze-dried powder were calculated according to the following formulae:

$$ITN = CtTN - C0TN \quad (6)$$

$$ITP = CtTP \times C0TP \quad (7)$$

where ITN and ITP are the increases in the TN and TP contents of *Gracilaria*, CtTN and CtTP represent the final TN and TP contents in *Gracilaria* freeze-dried powder, and C0TN and C0TP represent the initial TN and TP contents in each group of *Gracilaria* freeze-dried powder, respectively.

Excel software was used for linear fitting analysis of the data, and SPSS software (version 20.0) was used for one-way analysis of variance (ANOVA) to compare the differences in significance among the results from each group, with the significance level set at $p < 0.05$.

3. Results

3.1. Purification of Nitrogen and Phosphorus in Intensive Shrimp Culture Effluent by Sargassum Under Different Environmental Conditions

3.1.1. Purification of Nitrogen and Phosphorus in Intensive Shrimp Farming Effluent by Gracilaria with Different Biomass

(1) Changes in Basic Water Quality Indicators

The basic water quality indicators of each group were relatively stable, with salinity always maintained at (15 ± 1‰), temperature between 18–24 °C, pH between 8.0–9.0, and dissolved oxygen content between 6.00–7.30 $mg \cdot L^{-1}$.

(2) *Gracilaria* growth

The MBG group showed the highest rate of weight gain for *Gracilaria*, with the MBG group having a weight gain rate of 27.89%, and the HBG group having the 235th lowest

weight gain rate of 6.08%. After 18 days, the weight gain rate of the MBG group was 27.89%, while the HBG group had the lowest weight gain rate at 6.08% (Figure 1).

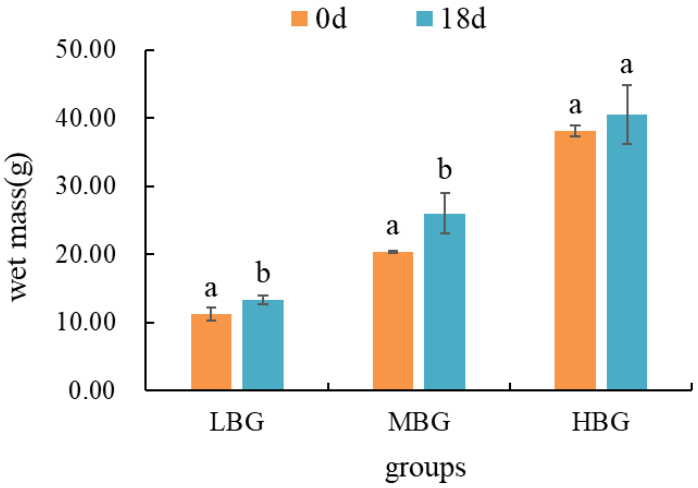

Figure 1. Changes in wet mass of *Gracilaria*. Different letters indicate significant differences between the same group ($p < 0.05$).

(3) Changes in TIN and PO_4^{3-}-P Concentrations in the Water

The HBG group achieved the best effluent TIN and PO_4^{3-}-P removal rates, and the amounts of TIN and PO_4^{3-}-P removed by the three groups did not form a complex relationship (Figure 2 and Table 1). After 18 days of the experiment, the NO_3^--N and TIN concentrations in the LBG, MBG, and HBG groups showed a continuous downward trend. The concentrations of NO_3^--N decreased from the initial 5.49 ± 0.24, 5.47 ± 0.36, and 5.40 ± 0.24 mg·L^{-1} to 4.56 ± 0.05, 3.24 ± 0.40, and 2.66 ± 0.41 mg·L^{-1} at 18 days, respectively, with the HBG group having the highest removal rate of 50.23%, which was higher than that of the MBG group (40.17%; $p < 0.05$), and both were significantly higher than that of the LBG group (18.65%). TIN decreased from the initial values of 5.59 ± 0.25, 5.58 ± 0.33, and 5.50 ± 0.27 mg·L^{-1} to 4.53 ± 0.03, 3.34 ± 0.37, and 2.79 ± 0.43 mg·L^{-1} at 18 days, respectively. The HBG group had the highest removal rate of 48.89%, which was higher than that of the MBG group (39.62%), and significantly higher than that of the LBG group (18.78%; $p < 0.05$). The levels of NH_4^+-N and NO_2^--N in each experimental group were low, both below 0.1 mg·L^{-1}. PO_4^{3-}-P also exhibited a continuous downward trend, decreasing from the initial values of 0.96 ± 0.05, 0.97 ± 0.04, and 0.98 ± 0.09 mg·L^{-1} to 0.39 ± 0.07, 0.03 ± 0.02, and 0.01 ± 0.00 mg·L^{-1} at 18 days, respectively, with the HBG group having the highest removal rate of 99.56%, higher than that of the MBG group (96.66%), which were both significantly higher than that of the LBG group (59.22%; $p < 0.05$).

Table 1. Purification of aquaculture effluent quality (removal rate %).

Item	LBG	MBG	HBG	Ctrl
NO_3^--N	18.65 ± 3.15 ^{b}	40.17 ± 10.87 ^{c}	50.23 ± 9.95 ^{c}	0.42 ± 4.05 ^{a}
TIN	18.78 ± 3.63 ^{b}	39.62 ± 9.94 ^{c}	48.89 ± 10.24 ^{c}	−0.22 ± 3.48 ^{a}
PO_4^{3-}-P	59.22 ± 6.49 ^{b}	96.66 ± 2.23 ^{c}	99.56 ± 0.26 ^{c}	15.54 ± 5.10 ^{a}

Note: Different letters within the same row indicate significant differences ($p < 0.05$).

In theory, under the same environmental factors and biomass variable conditions that are multiples of each other, the removal of nitrogen and phosphorus by *Gracilaria* in the effluent should be in a multiple relationship. However, this is not the case. *Gracilaria* at biomass conditions of 2 kg·m^{-3} and 4 kg·m^{-3} has the best absorption of nitrogen and phosphorus, and there is no significant difference, while the purification effect at 1 kg·m^{-3}

is lower, and there is no multiple relationship among the three groups. This indicates that under different biomass culture conditions, the purification of the same concentration of intensive shrimp farming effluent by *Gracilaria* is more rational at a biomass condition of 2 kg·m^{-3}, and a higher biomass is more conducive to the growth of *Gracilaria*.

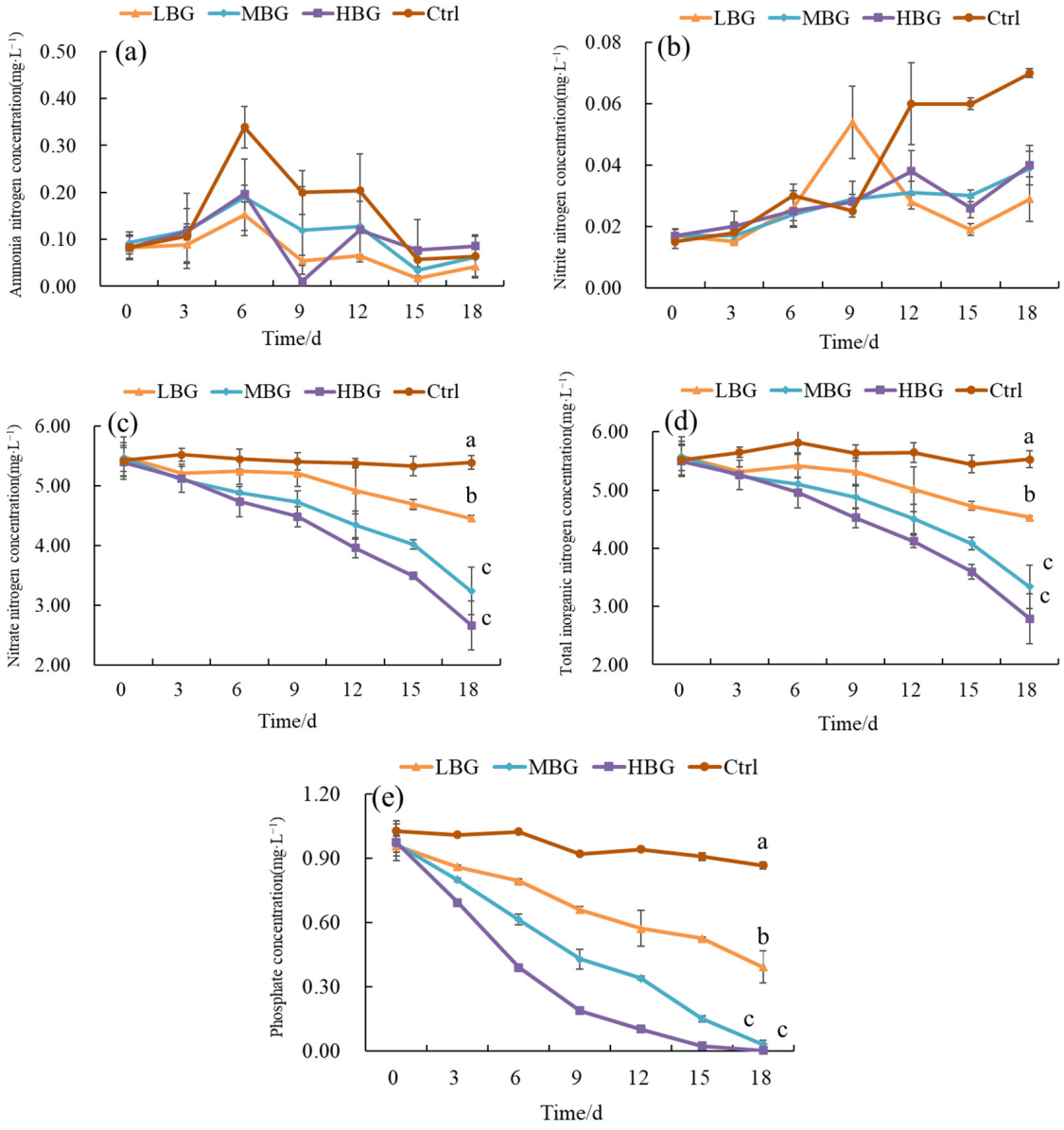

Figure 2. Variation in concentrations of inorganic N and P in aquaculture effluent. (**a**–**e**) represent the concentrations of NH_4^+-N, NO_2^--N, NO_3^--N, TIN, and PO_4^{3-}-P, respectively.

3.1.2. Purification of Nitrogen and Phosphorus in Intensive Shrimp Farming Effluent by Gracilaria Under Different Temperatures

(1) Changes in Basic Water Quality Indicators

The basic water quality indicators of each group were relatively stable, with salinity always maintained at 15 ± 1‰, pH between 8.0–9.1, and dissolved oxygen content between 5.70–7.50 mg·L^{-1}.

(2) Growth of *Gracilaria*

Gracilaria had the highest wet mass weight gain rate under a temperature of 25 °C. Among the groups, the 25 °C group had the highest weight gain rate of 20.64%, and the 20 °C group had the lowest weight gain rate of 13.05% (Figure 3).

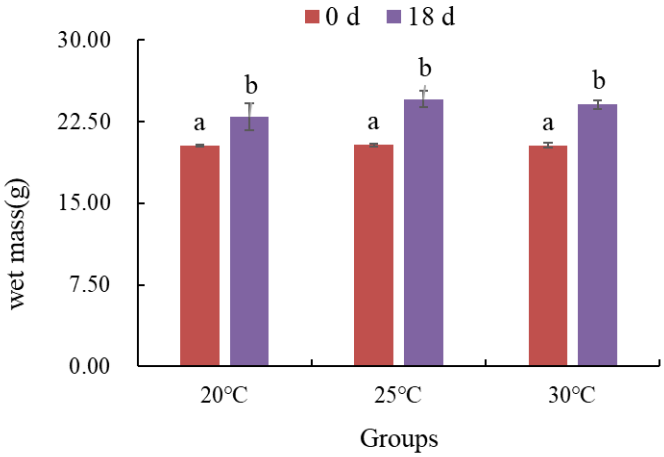

Figure 3. Changes in *Gracilaria* wet mass.

(3) Changes in TIN and PO_4^{3-}-P Concentrations in Water

The concentrations of NO_3^--N and TIN in the 20 and 25 °C groups showed a continuous downward trend, while the 30 °C group showed a trend of first increasing and then decreasing. There were no significant differences in the TIN removal rates among the three groups ($p > 0.05$), and the 30 °C group exhibited the highest PO_4^{3-}-P removal rate. NO_3^--N decreased from the initial values of 12.10 ± 0.41, 12.29 ± 0.44, and 12.05 ± 0.14 mg·L^{-1} to 9.82 ± 0.12, 9.89 ± 0.57, and 9.45 ± 0.91 mg·L^{-1} at 18 days, respectively, with the 30 °C group having the highest removal rate of 21.50%, which was higher than those of the 25 and 20 °C groups (19.35% and 18.75%, respectively), but there was no significant difference in the removal rate among the three groups ($p > 0.05$). TIN decreased from the initial values of 12.24 ± 0.41, 12.44 ± 0.43, and 12.20 ± 0.14 mg·L^{-1} to 10.00 ± 0.19, 10.03 ± 0.58, and 9.85 ± 0.73 mg·L^{-1} at 18 days, respectively. The removal rates were 18.18%, 19.18%, and 19.22%, respectively. PO_4^{3-}-P exhibited a continuous downward trend, decreasing from the initial values of 1.21 ± 0.01, 1.25 ± 0.01, and 1.23 ± 0.01 mg·L^{-1} to 0.30 ± 0.02, 0.06 ± 0.05, and 0.00 ± 0.00 mg·L^{-1} at 18 days, respectively, with the 30 °C group having a removal rate of 99.92%, which was significantly higher those of the 25 and 20 °C groups (95.17% and 75.11%, respectively; $p < 0.05$; Figure 4 and Table 2).

Table 2. Purification of aquaculture effluent (removal rate %).

Item	20 °C	25 °C	30 °C	Ctrl
NO_3^--N	18.75 ± 3.03 ^{b}	19.35 ± 6.76 ^{b}	21.50 ± 8.47 ^{b}	3.54 ± 1.69 ^{a}
TIN	18.18 ± 2.77 ^{b}	19.18 ± 6.54 ^{b}	19.22 ± 6.93 ^{b}	1.70 ± 0.86 ^{a}
PO_4^{3-}-P	75.11 ± 1.95 ^{b}	95.17 ± 3.70 ^{c}	99.92 ± 0.00 ^{d}	4.44 ± 0.32 ^{a}

Gracilaria has no significant effect on the purification of the same concentration of inorganic nitrogen within the water temperature range of 20–30 °C, and the TIN removal rates of the 20 °C, 25 °C, and 30 °C groups can reach 18.18%, 19.18%, and 19.22%, respectively. However, the purification of PO_4^{3-}-P will increase with the increase of temperature, and the removal rates are 75.11%, 95.17%, and 99.92%, respectively. This indicates that suitable water temperature conditions can effectively affect the growth of *Gracilaria* and the absorption efficiency of phosphorus in the effluent.

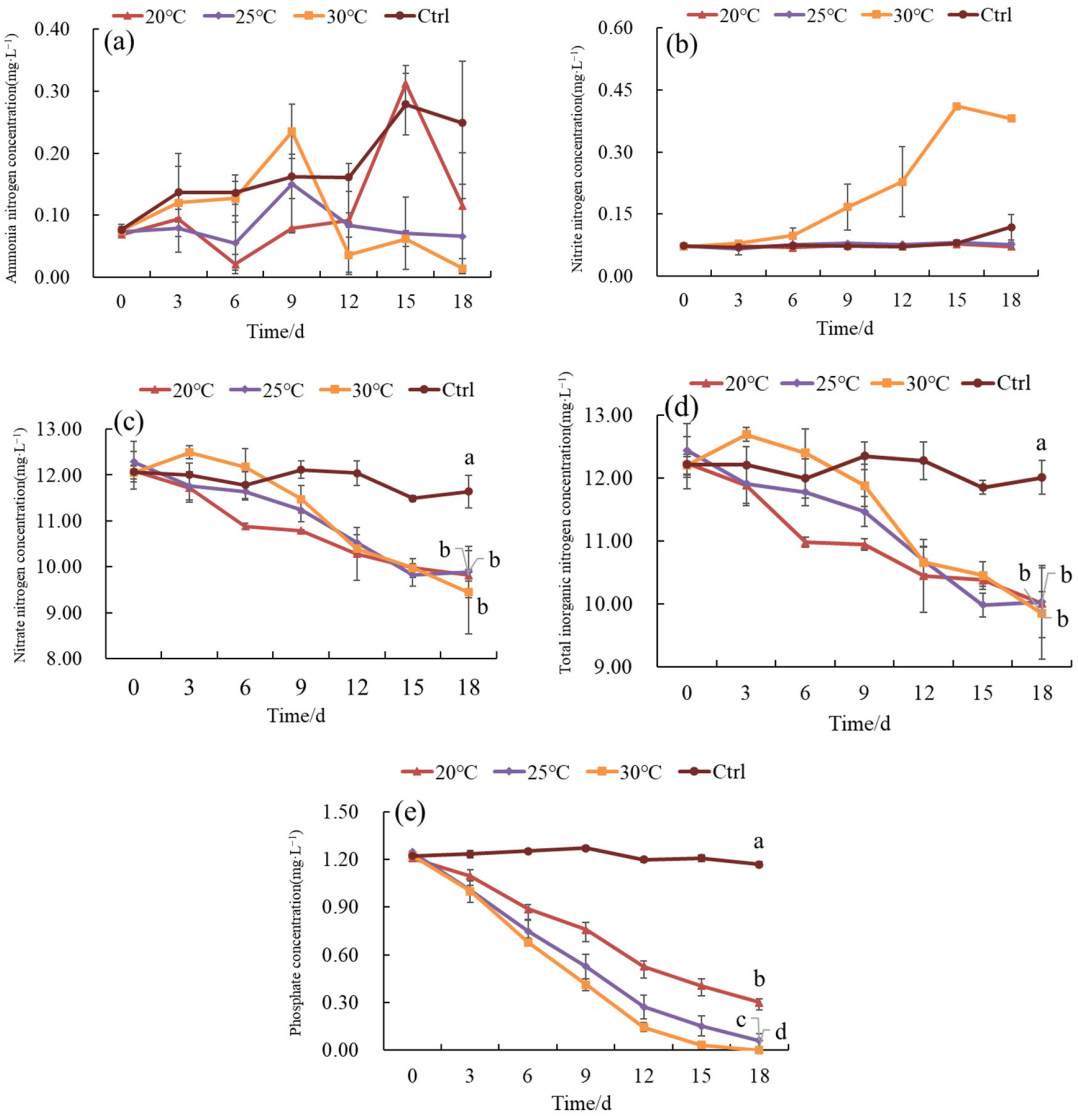

Figure 4. Variation in inorganic N and P concentrations in aquaculture effluent.

3.1.3. Purification of Nitrogen and Phosphorus in Intensive Shrimp Farming Effluent by Gracilaria Under Different Salinity Conditions

(1) Changes in Basic Water Quality Indicators

The dissolved oxygen content in each group of water was stable between 6.00–7.00, the temperature was stable between 25–28 °C, and the pH varied between groups with different salinities. The lower the salinity, the greater the pH change; however, it was maintained between 7.6–9.4, all within the pH range required for *Gracilaria* growth.

(2) *Gracilaria* Growth and Survival

The salinity of 5‰ exhibited a negative effect on the growth of *Gracilaria*, while the highest weight gain rate was observed for *Gracilaria* at a salinity of 20‰. By day 18 of the experiment, most of the *Gracilaria* at salinity 5‰ was transparent and white and had dissolved, whereas some of the *Gracilaria* at salinity 10‰ had transparent tips, and the *Gracilaria* at salinities 20 and 30‰ survived. The salinity 20‰ group had the highest weight gain rate of 12.90%, and the salinity 5‰ group had the lowest weight gain rate of −1.26% (Figures 5 and 6). Within the salinity range of 5–30‰, low salinity had a significant effect on the survival of *Gracilaria*. At salinities of 5 and 10‰, *Gracilaria* exhibited varying degrees of whitening and dissolution; the lower the salinity, the greater the impact. This indicates that *Gracilaria* can adapt to brackish water or seawater with a salinity of 10‰ or above.

Figure 5. Status of *Gracilaria* under different salinities.

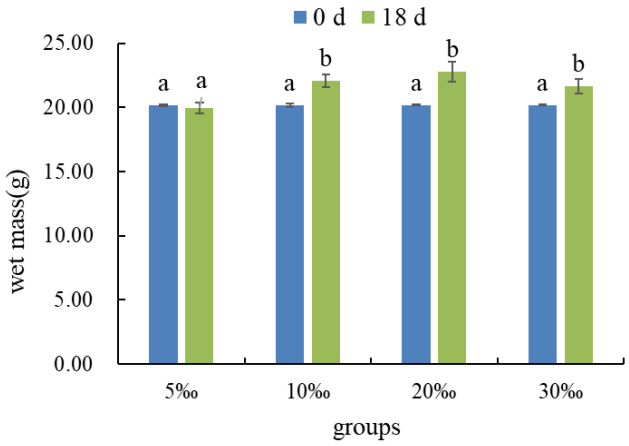

Figure 6. Changes in wet mass of *Gracilaria*.

(3) Changes in N and P Concentrations in Water

The salinity 20‰ group achieved the best TIN and PO_4^{3-}-P removal rates. The NO_3^--N and TIN concentrations in the salinity 5‰ group first decreased, then increased, and then decreased again, while they exhibited a continuous downward trend in the salinity 10, 20, and 30‰ groups. NO_3^--N decreased from the initial values of 11.65 ± 0.29, 11.74 ± 0.12, 11.85 ± 0.27, and 11.40 ± 0.29 mg·L^{-1} to 10.22 ± 0.18, 9.51 ± 0.25, 8.90 ± 0.36, and 8.95 ± 0.35 mg·L^{-1} at 18 days, respectively. The salinity 20‰ group had the highest removal rate of 24.78%, which was higher than the rates achieved by the salinity 30‰ (21.51%) and 10‰ (18.61%) groups, but there was no significant difference among them ($p > 0.05$). The removal rates for these three groups were significantly higher than that achieved by the salinity 5‰ group (12.23%; $p < 0.05$). TIN decreased from the initial values of 11.74 ± 0.25, 11.80 ± 0.14, 11.99 ± 0.24, and 11.60 ± 0.38 mg·L^{-1} to 10.34 ± 0.16, 10.09 ± 0.27, 9.05 ± 0.38, and 9.09 ± 0.35 mg·L^{-1} at 18 days, respectively. The salinity 20‰ group achieved the highest removal rate of 24.45%, which was higher than those of the

salinity 30‰ (21.59%) and 10‰ (18.26%) groups, but there was no significant difference among them ($p > 0.05$); however, these values were significantly higher than that achieved by the salinity 5‰ group (11.88%; $p < 0.05$). The concentrations of NH_4^+-N and NO_2^--N in each experimental group were low, with NH_4^+-N below 0.24 mg·L^{-1} and NO_2^--N below 0.12 mg·L^{-1}. The PO_4^{3-}-P concentration of each experimental group showed a continuous downward trend, decreasing from the initial values of 1.32 ± 0.03, 1.32 ± 0.01, 1.36 ± 0.01, and 1.34 ± 0.01 mg·L^{-1} to 0.62 ± 0.05, 0.12 ± 0.03, 0.07 ± 0.00, and 0.10 ± 0.03 mg·L^{-1} at 18 days, respectively. The salinity 20‰ group achieved the highest removal rate of 95.00%, which was higher than those of the salinity 30‰ (92.95%) and 10‰ (91.13%) groups. There was no significant difference among the three groups ($p > 0.05$); however, their removal rates were all significantly higher than that of the salinity 5‰ group (53.33%; $p < 0.05$; Figure 7 and Table 3). Additionally, the low-salinity condition of 5‰ may have caused the lowest TIN and PO_4^{3-}-P removal rates due to damage to the algae segments, resulting in a negative effect on *Gracilaria*. The weight gain rate of *Gracilaria* at a salinity of 20‰ was the highest (12.90%), and the N and P removal rates were the highest (24.78% and 95.00%, respectively). From the results of the moderate biomass and temperature experiments, it can be concluded that the growth and survival of *Gracilaria* used in this study are inhibited under low-salinity conditions. The weight gain effect was the best at a salinity of 15‰, and the weight gain rate of *Gracilaria* first increased and then decreased with an increase in salinity within the salinity range of 10–30‰.

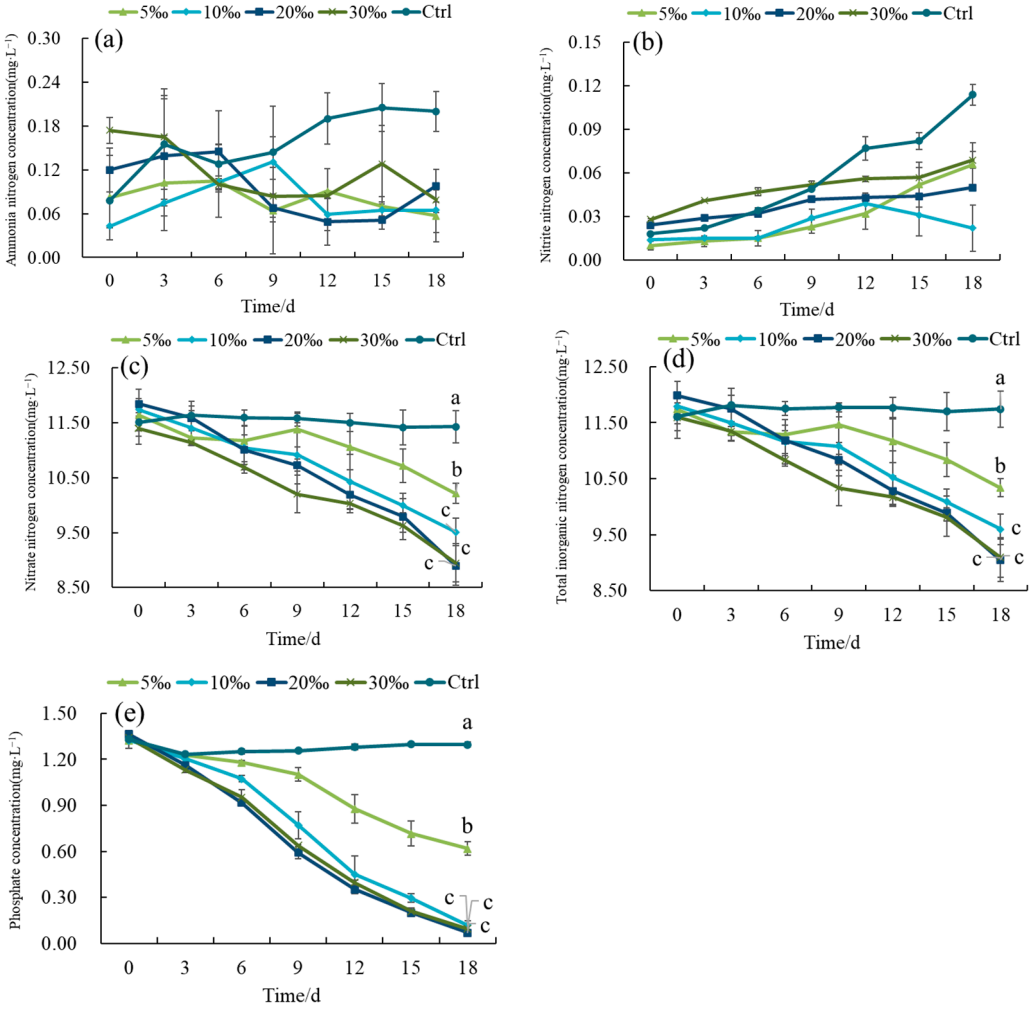

Figure 7. Variations in inorganic N and P concentrations in aquaculture effluent.

Table 3. Purification of aquaculture effluent quality (removal rate %).

Item	5	10	20	30	Ctrl
NO_3^--N	12.23 ± 2.56 ^{b}	18.61 ± 2.99 ^{bc}	24.78 ± 4.54 ^{c}	21.51 ± 1.40 ^{c}	0.61 ± 4.72 ^{a}
TIN	11.88 ± 2.59 ^{b}	18.26 ± 3.30 ^{bc}	24.45 ± 4.54 ^{c}	21.59 ± 0.97 ^{c}	−1.27 ± 4.97 ^{a}
PO_4^{3-}-P	53.33 ± 2.84 ^{b}	91.13 ± 2.25 ^{c}	95.00 ± 0.18 ^{c}	92.95 ± 1.80 ^{c}	2.40 ± 3.70 ^{a}

3.2. Purification of Intensive Shrimp Farming Effluent by Gracilaria Coupled with Oysters

3.2.1. Changes in Water Quality Indicators of Intensive Shrimp Farming Effluent

(1) Changes in Conventional Physicochemical Factors of Intensive Shrimp Farming Effluent

During the experiment coupling oyster with *Gracilaria*, the environment was relatively stable, with a temperature, dissolved oxygen concentration, pH value, and salinity of 25 ± 3 °C, 7.0 ± 0.5 mg·L^{-1}, 8.3 ± 0.5, and 15 ± 1‰, respectively, all meeting the normal growth requirements of *Gracilaria* and oysters.

(2) Changes in Nutrient Concentrations of Intensive Shrimp Farming Effluent

The coupling of oysters and *Gracilaria* had a significant purification effect on TIN and PO_4^{3-}-P in the intensive shrimp farming effluent (Figure 8 and Table 4), and there was a significant difference in the TP removal rate ($p < 0.05$). After 48 days of the experiment, the TIN and PO_4^{3-}-P concentrations exhibited a downward trend during the experiment, decreasing from the initial values of 18.26 ± 0.48 and 1.65 ± 0.06 mg·L^{-1} to 7.39 ± 1.44 and 0.04 ± 0.03 mg·L^{-1} after 48 days, respectively, with removal rates of 59.56% and 97.43%, significantly higher than those of the Ctrl group (38.29% and 3.47%; $p < 0.05$). The TN and TP concentrations decreased from the initial values of 31.66 ± 3.14 and 2.80 ± 0.28 mg·L^{-1} to 11.50 ± 2.76 and 0.66 ± 0.05 mg·L^{-1} at 35 days, with removal rates of 63.67% and 76.25%, respectively, which were significantly higher than those of the Ctrl group (38.29% and 35.61%; $p < 0.05$).

Table 4. Purification of aquaculture effluent quality (removal rate %).

Item	CSG	Ctrl
TIN	59.56 ± 7.54 ^{a}	38.29 ± 9.68 ^{b}
PO_4^{3-}-P	97.43 ± 1.86 ^{a}	3.47 ± 5.91 ^{b}
TN	62.56 ± 11.66 ^{a}	52.54 ± 5.34 ^{a}
TP	75.90 ± 3.77 ^{a}	29.85 ± 2.87 ^{b}

The TSS concentrations in the CSG and Ctrl groups showed a fluctuating upward trend, with that in the CSG group increasing from the initial value of 42.67 ± 0.94 mg·L^{-1} to 84.00 ± 16.06 mg·L^{-1}; however, it was lower than that of the Ctrl group (101.67 ± 18.08 mg·L^{-1}), and there was no significant difference between the two groups ($p > 0.05$). This indicates that the CSG group had an inhibitory effect on the TSS concentration.

The trend of the change in the COD concentration change in the CSG group was the same as that of TSS, increasing from the initial value of 6.74 ± 0.35 mg·L^{-1} to 15.51 ± 2.05 mg·L^{-1}, while that of the Ctrl group increased to 14.25 ± 2.29 mg·L^{-1}, and there was no significant difference in concentration between the two groups at 48 days ($p > 0.05$).

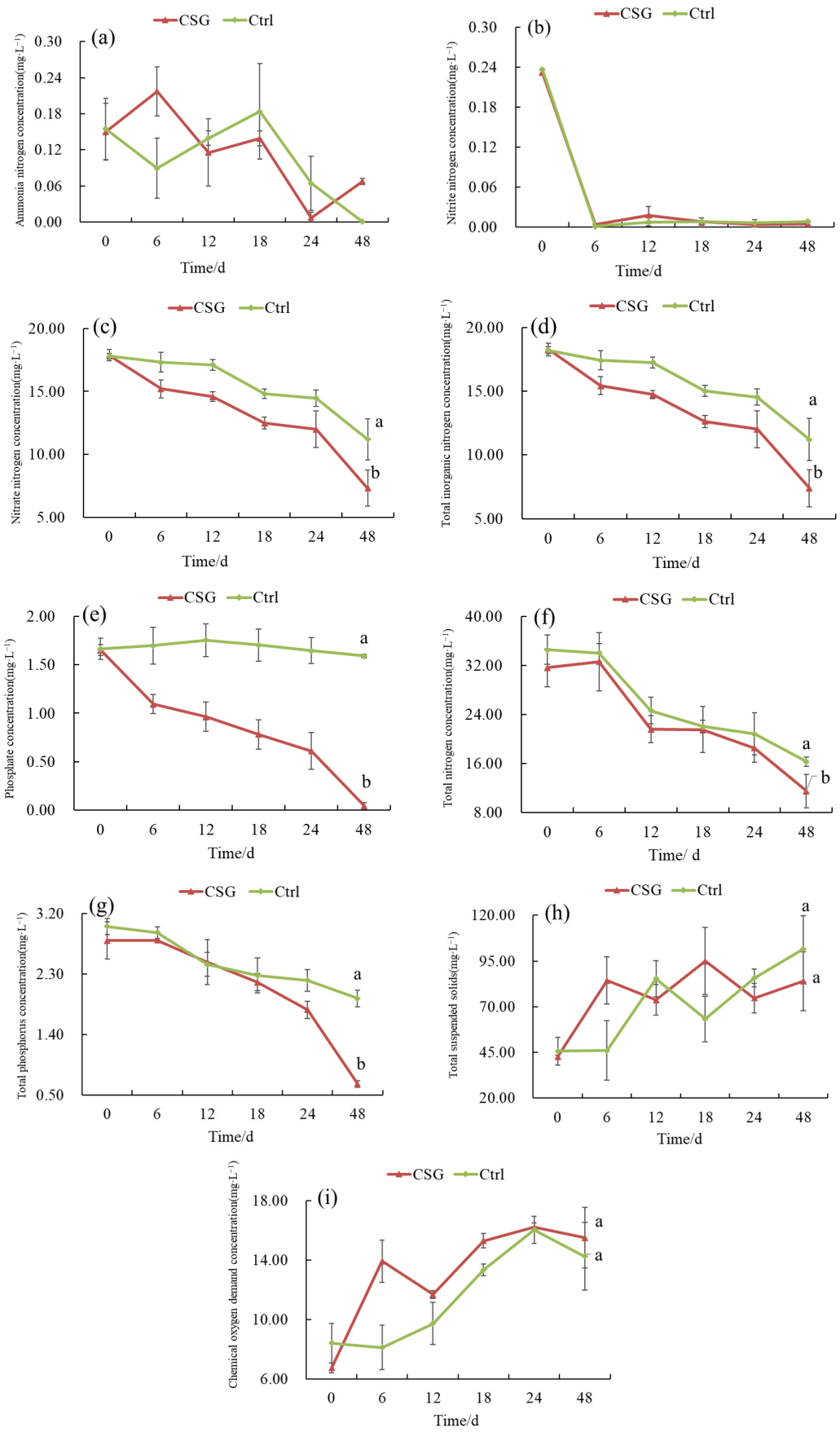

Figure 8. Variation in N and P concentrations in aquaculture effluent. (**a**–**i**) represent the concentrations of NH_4^+-N, NO_2^--N, NO_3^--N, TIN, PO_4^{3-}-P, TN, TP, TSS, and COD, respectively.

3.2.2. Survival and Growth of Oysters

After 48 days of the experiment, the survival rate of oysters in the CSG group was 62.22%. The weight of oysters increased from the initial 120.87 ± 18.13 g to 121.65 ± 16.40 g at 48 days, with a weight gain rate of 0.64%, and there was no significant difference in the weight gain rate before and after the experiment ($p > 0.05$) (Figure 9).

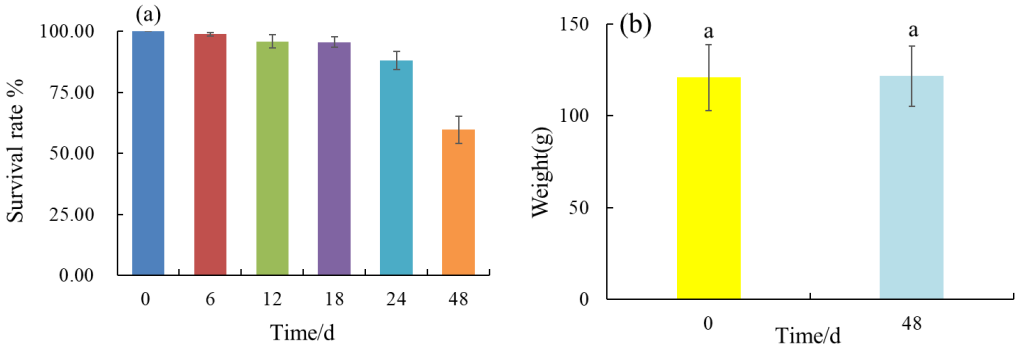

Figure 9. Growth and survival of *hongkongensis*. (**a**,**b**) show the survival rate and weight of oysters, respectively.

3.2.3. Changes in Wet Mass of Gracilaria

After 48 days, the wet mass of *Gracilaria* increased from the initial 1.60 ± 0.01 kg to 2.10 ± 0.04 kg at 48 days, with a weight gain rate of 31.01% (Figure 10).

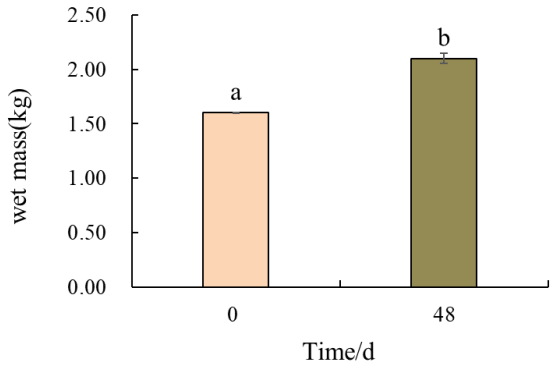

Figure 10. *Gracilaria* growth.

3.3. Deposition Effects of Nitrogen and Phosphorus in Effluent by Gracilaria Coupled with Oyster

3.3.1. Changes in Dry Weight, Content per Unit Dry Weight, and Net Increase per Unit Dry Weight of Gracilaria

The content per unit dry weight of *Gracilaria* was highest under a biomass of 2 kg·m^{-3}, temperature of 30 °C, and salinity of 30‰, which were 106.38 ± 5.86, 88.83 ± 2.78, and 99.17 ± 4.67 mg·g^{-1}, respectively. Under laboratory conditions, the content per unit dry weight of *Gracilaria* in a normal survival state exhibited an upward trend, whereas the content per unit dry weight of *Gracilaria* in the oyster and *Gracilaria* coupling exhibited the opposite experimental results (Table 5).

On day 0, the contents per unit dry weight of *Gracilaria* in the biomass, temperature, and salinity groups were 86.97 ± 4.5, 69.06 ± 1.72, and 79.21 ± 2.00 mg·g^{-1}, respectively. On day 18, the contents per unit dry weight of the biomass 1, 2, and 4 kg·m^{-3} groups were 101.79 ± 3.75, 106.38 ± 5.86, and 90.45 ± 2.82 mg·g^{-1}, respectively, with the 2 kg·m^{-3} group exhibiting the highest content per unit dry weight. Additionally, the net increase per unit dry weight of this group was 19.66 ± 5.86 mg·g^{-1}, which was significantly higher than those of the 1 and 4 kg·m^{-3} groups (9.16 ± 3.75 and 8.89 ± 2.82 mg·g^{-1}, respectively;

$p < 0.05$). The contents per unit dry weight of the temperature 20, 25, and 30 °C groups were 74.83 ± 2.94, 76.26 ± 3.05, and 88.83 ± 2.78 mg·g^{-1}, respectively, with the 30 °C group having the highest content per unit dry weight, and the net increase per unit dry weight was 19.74 ± 2.78 mg·g^{-1}, which was significantly higher than those of the 20 and 25 °C groups (5.74 ± 2.94 and 7.17 ± 3.05 mg·g^{-1}, respectively; $p < 0.05$). The contents per unit dry weight of the salinity 5, 10, 20, and 30‰ groups were 61.93 ± 3.13, 84.57 ± 2.50, 93.21 ± 4.47, and 99.17 ± 4.67 mg·g^{-1}, respectively, with the salinity 30‰ group having the highest content and net increase per unit dry weight. The net increase per unit dry weight was 19.96 ± 4.67 mg·g^{-1}, significantly higher than those of the salinity 5 and 10‰ groups (−17.28 ± 3.13 and 5.36 ± 2.50 mg·g^{-1}, respectively; $p < 0.05$), and there was no significant difference with the salinity 20‰ group ($p > 0.05$). There was no significant difference in the content per unit dry weight of *Gracilaria* before and after the coupling of oysters and *Gracilaria* for 48 days ($p > 0.05$), and the net increase per unit dry weight of *Gracilaria* decreased to −7.77 ± 3.13 mg·g^{-1}.

Table 5. Moisture content change, dry weight ratio, and net dry weight increment of *Gracilaria*.

Groups	Dry Weight (g)	Content per Unit Dry Weight (mg·g^{-1})	Net Increase per Unit Dry Weight (mg·g^{-1})
BG_0	1.75 ± 0.09 (20 g)	86.97 ± 4.50	
LBG_{18}	1.36 ± 0.05 (10 g)	101.79 ± 3.75	9.16 ± 3.75 ^{a}
MBG_{18}	2.79 ± 0.47 (20 g)	106.38 ± 5.86	19.66 ± 5.86 ^{b}
HBG_{18}	3.67 ± 0.45 (40 g)	90.45 ± 2.82	8.89 ± 2.82 ^{a}
T_0	1.39 ± 0.04	69.06 ± 1.72	
20 °C_{18}	1.72 ± 0.13	74.83 ± 2.94	5.74 ± 2.94 ^{a}
25 °C_{18}	1.87 ± 0.11	76.26 ± 3.05	7.17 ± 3.05 ^{a}
30 °C_{18}	2.14 ± 0.10	88.83 ± 2.78	19.74 ± 2.78 ^{b}
S_0	1.60 ± 0.04	79.21 ± 2.00	
5_{18}	1.23 ± 0.05	61.93 ± 3.13	−17.28 ± 3.13 ^{a}
10_{18}	1.87 ± 0.06	84.57 ± 2.50	5.36 ± 2.50 ^{b}
20_{18}	2.12 ± 0.12	93.21 ± 4.47	14.00 ± 4.47 ^{c}
30_{18}	2.15 ± 0.12	99.17 ± 4.67	19.96 ± 4.67 ^{c}
CSG_0	2.72 ± 0.11	135.97 ± 6.39	
CSG_{48}	2.57 ± 0.21	128.20 ± 1.13	−7.77 ± 1.13 ^{a}

Note: Different superscript letters indicate significant differences between different groups in the experiment ($p < 0.05$).

3.3.2. Total Nitrogen and Phosphorus Content and Biological Deposition of Dry Matter of Gracilaria

The TN content of *Gracilaria* dry matter was the highest under the biomass 1 kg·m^{-3}, and the TP content was the highest under biomass 2 kg·m^{-3}, with the highest increase in the TN and TP contents. There was no significant difference in the total nitrogen content of dry matter at temperatures of 20–30 °C ($p > 0.05$), and the total phosphorus content increased significantly with higher temperatures. There was no significant difference in the TN and TP contents of *Gracilaria* dry matter at salinities of 10–30‰ ($p > 0.05$) (Table 6).

On day 18, the TN contents of the biomass 1, 2, and 4 kg·m^{-3} groups were 19.35 ± 3.72, 17.57 ± 2.38, and 12.55 ± 1.76 mg·g^{-1}, respectively, and the TP contents were 5.38 ± 0.34, 5.87 ± 0.91, and 5.58 ± 0.84 mg·g^{-1}, respectively. The 2 kg·m^{-3} group exhibited the highest increases in the TN and TP contents of dry matter, which were 6.90 and 2.97 mg·g^{-1}, higher than those of the 1 kg·m^{-3} group (6.17 and 2.48 mg·g^{-1}, respectively) and the 4 kg·m^{-3} group (−0.84 and 2.68 mg·g^{-1}, respectively). The TN contents of the temperature 20, 25, and 30 °C groups were 16.56 ± 1.31, 20.01 ± 2.71, and 15.89 ± 1.53 mg·g^{-1}, respectively, and the TP contents were 4.27 ± 0.69, 5.74 ± 0.28, and 5.92 ± 0.72 mg·g^{-1}, respectively. There was no significant difference in the increase in TN content among the three groups

($p > 0.05$), and the increase in the TP content at 30 °C was significantly higher than those at 20 and 25 °C ($p < 0.05$). The increase in the TN content at 25 °C was the highest, at 3.54 mg·g^{-1}, and the increase in the TP content at 30 °C was the highest, at 2.85 mg·g^{-1}. The TN and contents of *Gracilaria* dry matter at salinities of 5, 10, 20, and 30‰ were 13.95 ± 2.89, 18.28 ± 3.33, 17.02 ± 2.45, and 18.08 ± 1.42 mg·g^{-1}, respectively, and the TP contents were 5.56 ± 1.30, 6.24 ± 0.39, 5.96 ± 0.73, and 5.59 ± 0.55 mg·g^{-1}, respectively. There was no significant difference in the increases in the TN and TP contents among the salinity 5–30‰ groups ($p > 0.05$), and the increases at salinity 10‰ were the highest, reaching 1.53 and 3.85 mg·g^{-1}, respectively. After the coupling of oysters and *Gracilaria* for 48 days, the TN and TP contents of *Gracilaria* dry matter increased significantly ($p < 0.05$), from 23.99 ± 2.15 and 2.49 ± 0.21 mg·g^{-1} to 38.36 ± 10.37 and 8.54 ± 0.86 mg·g^{-1}, which are increases of 14.37 and 6.29 mg·g^{-1}, respectively.

Table 6. Total nitrogen and phosphorus contents and biological deposition of *Gracilaria* dry matter.

Groups	Total Nitrogen Content (mg·g^{-1})	Increase in Total Nitrogen Content (mg·g^{-1})	Total Phosphorus Content (mg·g^{-1})	Increase in Total Phosphorus Content (mg·g^{-1})
BG_0	12.42 ± 1.24 ^{a} (20 g)		2.94 ± 0.26 ^{a} (20 g)	
LBG_{18}	19.35 ± 3.72 ^{b}	6.17 ± 3.72 ^{b}	5.38 ± 0.34 ^{b}	2.48 ± 0.34 ^{a}
MBG_{18}	17.57 ± 2.38 ^{b}	6.90 ± 2.38 ^{b}	5.87 ± 0.91 ^{b}	2.97 ± 0.91 ^{a}
HBG_{18}	12.55 ± 1.76 ^{a}	−0.84 ± 1.76 ^{a}	5.58 ± 0.84 ^{b}	2.68 ± 0.84 ^{a}
T_0	16.47 ± 1.83 ^{a}		3.07 ± 0.47 ^{a}	
20 °C_{18}	16.56 ± 1.31 ^{a}	0.09 ± 1.31 ^{b}	4.27 ± 0.69 ^{b}	1.20 ± 0.69 ^{a}
25 °C_{18}	20.01 ± 2.71 ^{a}	3.54 ± 2.71 ^{b}	5.74 ± 0.28 ^{c}	2.67 ± 0.28 ^{b}
30 °C_{18}	15.89 ± 1.53 ^{a}	−0.58 ± 1.53 ^{b}	5.92 ± 0.72 ^{c}	2.85 ± 0.72 ^{b}
S_0	16.75 ± 1.39 ^{a}		2.39 ± 0.51 ^{a}	
5_{18}	13.95 ± 2.89 ^{a}	−2.80 ± 2.89 ^{a}	5.56 ± 1.30 ^{b}	3.17 ± 1.30 ^{a}
10_{18}	18.28 ± 3.33 ^{a}	1.53 ± 3.33 ^{a}	6.24 ± 0.39 ^{b}	3.85 ± 0.39 ^{a}
20_{18}	17.02 ± 2.45 ^{a}	0.27 ± 2.45 ^{a}	5.96 ± 0.73 ^{b}	3.57 ± 0.73 ^{a}
30_{18}	18.08 ± 1.42 ^{a}	1.33 ± 1.42 ^{a}	5.59 ± 0.55 ^{b}	3.20 ± 0.55 ^{a}
CSG_0	23.99 ± 2.15 ^{a}		2.49 ± 0.21 ^{a}	
CSG_{48}	38.36 ± 10.37 ^{b}	14.37 ± 10.37	8.54 ± 0.86 ^{b}	6.29 ± 0.86

Note: Different superscripts indicate significant differences between different groups in the experiment ($p < 0.05$).

4. Discussion

4.1. Impact of Biomass Parameters on the Purification of Intensive Shrimp Farming Effluent by Gracilaria

As the distribution of soluble nutrients in water is relatively uniform, plants with different growth types and individuals of the same species may be more inclined to compete for resources, such as living space and light [34]. A species can allocate its resources under certain biomass limitations. Once this limit is exceeded, growth inhibition or other negative effects occur [35]. In this study, *Gracilaria* was found to survive and grow normally under three different biomass conditions, and a biomass of 4 kg·m^{-3} or below did not affect the survival of *Gracilaria*. The effect of density on the growth of *Gracilaria* is complex and linked to both environmental stress and nutrient availability. In this study, *Gracilaria* grew fastest at 2 kg·m^{-3}. This density likely provides sufficient light, oxygen, and nutrients, while avoiding excessive competition. However, higher densities may lead to competition-induced issues, such as light shortage and poor water flow, negatively affecting growth, despite potentially providing more nutrients [36]. Moreover, mutual shading and competition among *Gracilaria* individuals at high densities may restrict effective nutrient absorption, thereby influencing growth rates and biomass accumulation [37]. Studies have found that, within a certain density range, the growth rate and biomass yield of *Gracilaria* are positively correlated with density. However, once the critical point is exceeded, the growth rate tends

to plateau or even decline, which is consistent with the phenomena observed in this study. Additionally, some studies have reported that the absorption efficiency of nutrients, such as N and P, by *Gracilaria* varies at different densities and that its absorption capacity is closely related to the nutrient concentration of the water [38]. These findings enhance our understanding of how density affects *Gracilaria* growth and nutrient absorption and underpin our study theoretically.

4.2. Impact of Temperature Conditions on the Purification of Intensive Shrimp Farming Effluent by Gracilaria

The adaptations of Macroalgae to temperature differ among species. Excessively high or low temperatures and light intensity inhibit algal growth [39]. In this study, within a range of 20–30 °C, water temperature has no significant effect on *Gracilaria* survival. However, the weight gain rate of *Gracilaria* is significantly different under different water temperature conditions, and the highest weight gain rate was observed at a water temperature of 25 °C, reaching 20.64%. Huang [40] revealed the molecular mechanism driving the adaptation of *Gracilaria* bailinae to high temperatures through transcriptome analysis. Other studies have also explored the impact of temperature on the growth, physiology, and molecular aspects of *Gracilaria*. Dawes explored the response of the tropical red seaweed *Gracilaria* cornea to temperature, salinity, and light, and revealed that they significantly impacted its growth. This study was similar to ours in that it focused on the effect of temperature on *Gracilaria* growth within a specific range, but it differed in that it also incorporated the comprehensive influences of salinity and light, unlike our exclusive focus on the effect of temperature on weight gain rate and survival; no significant impact on survival was observed, but there were significant differences in weight gain rate within 20–30 °C [41]. Schneider explored the effects of UV-visible radiation on *Gracilaria cornea* growth, photosynthesis, pigment accumulation, and UV-absorbing compounds. Unlike our temperature-focused study on survival and weight gain rates, the authors examined the effects of light radiation, including UV and visible light, on the physiological and biochemical changes in the algae [42]. Borlongan assessed the effects of light quality and temperature on photosynthesis and pigment content in the subtidal edible red algae *Meristotheca papulosa* in Japan. While both studies examined the effect of temperature on algal photosynthesis and growth, they differed in that Borlongan's study involved a different species and included light quality, whereas this study focused on the effect of temperature on the weight gain rate of *Gracilaria* [43].

4.3. Impact of Salinity Conditions on the Purification of Intensive Shrimp Farming Effluent by Gracilaria

Salinity is an important environmental factor that affects algal growth and distribution. Salinity affects the absorption of N and P by affecting the osmotic pressure inside and outside plant cells. Macroalgae have a certain adaptability to salinity changes owing to environmental changes in the intertidal area where they naturally survive, with differences in survival and growth under different salinities [44]. In the present study, *Gracilaria* displayed differential bleaching and dissolution at salinities of 5 and 10‰, with more pronounced adverse effects evident at lower salinity levels. This observation implies that the growth and survival of *Gracilaria* are compromised under low-salinity conditions, a finding that aligns with Martins' research on salinity-induced growth modulation in *Gracilaria*. The detrimental impacts of low salinity may be attributed to segmental damage in the algae, which, in turn, exerts a negative influence on the growth trajectory of *Gracilaria* [45]. However, in another study on the stress of low salinity on *U. pinnatifida* by She [46], it was found that short-term low-salinity stress did not have adverse effects on algal segments, but prolonged stress caused irreversible functional loss.

4.4. Purification Effects of Gracilaria Coupled with Oysters on Effluent

In aquaculture, tailwater treatment is crucial for environmental sustainability and operational benefits. Various treatment methods have unique pros and cons [47]. Sedimentation is widely used to eliminate large-particle suspended solids and certain pollutants like NH_4^+-N, NO_2^--N, PO_4^{3-}-P, and NO_3^--N. However, it is less effective on small-particle suspended solids. Regular bottom sludge cleaning is necessary to prevent hydrogen sulfide generation. The sedimentation tank's hydraulic retention time and flow velocity significantly impact treatment effectiveness and require careful control [48]. Ozone oxidation excels in sterilization and disinfection, unaffected by tailwater turbidity, and can eliminate pathogenic bacteria that ultraviolet light misses. Yet, ozone dosage must be strictly regulated to avoid air pollution and water residue, which could harm fish health [49]. Electrochemical technology removes nitrogen, phosphorus, and organic matter through oxidation-reduction reactions. It offers advantages like simple equipment, high efficiency, and small size, with additional capabilities in water sterilization and drug residue detection. However, it has high equipment costs, and operational parameters such as current density, electrode spacing, and electrolysis time need precise control for optimal results [50]. Constructed wetlands are cost-effective and easy to maintain, effectively removing nitrogen, phosphorus, organic matter, and suspended solids. Different wetland types yield varying treatment outcomes; for instance, vertical flow constructed wetlands are most effective for TN removal. Their efficiency is influenced by hydraulic retention time, season, pollution levels, and other factors [51]. Microbial agents can eliminate NH_4^+-N, COD, and NO_2^--N from tailwater and inhibit Vibrio growth. The choice of microbial agent should be based on the types and concentrations of pollutants in the tailwater [52]. Biological filters convert NH_4^+-N to NO_3^--N via nitrification, showing good NH_4+-N removal, but poor COD removal [53]. Constructed wetlands, microbial agents, and biological filters are all technologies that utilize the absorption, metabolism, and degradation actions of aquatic plants, animals, and microorganisms to remove pollutants. This is consistent with the purification mechanism of *Gracilaria*, all of which are based on the physiological and ecological characteristics of organisms to achieve the removal of pollutants. In conclusion, each tailwater treatment method in aquaculture has distinct advantages and limitations. Practical application requires a comprehensive selection of suitable methods considering factors like aquaculture scale, tailwater characteristics, and environmental requirements to achieve the best treatment and environmental outcomes.

Numerous studies have been conducted on the purification of aquaculture effluent using oysters and *Gracilaria* [54–58], and there have also been reports on the purification of effluent by co-culturing bivalves and Macroalgae [59]. In this study, the coupling of *Gracilaria* and oysters had a good purification effect on water quality, with TIN and PO_4^{3-}-P removal rates of 59.56% and 97.43%, respectively, and TN and TP removal rates of 63.67% and 76.25%, respectively, with no significant effect on TSS removal. In Jones' study, the combination of oysters and *Gracilaria* effectively removed TSS [20]. However, in this study, oysters exerted no evident effect on TSS removal. This difference may have resulted from the types of oysters, experimental conditions, and nature of the TSS. Studies have shown that sedimentation, oyster filtration, and macroalgal uptake can remove N and P from shrimp farm wastewater. The NH_4^+-N and NO_3^--N removal rates were 76% and 30%, respectively, whereas the TP removal rate was 14%. There are certain differences between the two results, which may have been related to the different test water body background environment and biological selection. The effect of water quality purification by the coupling of oysters and *Gracilaria* in this study was better. In this study, the oysters may have been in a slow growth or dormant phase, decreasing their TSS removal effect. In addition, oyster density can influence TSS removal. In low-density farming, oysters

may not consume sufficient particles because of weak competition. Moreover, the filter-feeding habits of oysters affect TSS removal. Different oyster species have varying filter-feeding efficiencies and particle selectivities, which might have led to the insignificant TSS removal by oysters in this study [60]. As a common macroalgae, *Gracilaria* may positively affect TSS removal as its biomass grows and it adsorbs and deposits particles in water. It absorbs nutrients and, owing to its complex structure, suspended particles, thus reducing TSS and improving water quality. Increasing the *Gracilaria* biomass further boosted the adsorption capacity. In this study, the *Gracilaria*–oyster combination likely compensated for the limited TSS removal by oysters, enhancing the system's TSS removal efficiency through adsorption and deposition [61]. In summary, the insignificant TSS removal by oysters can be attributed to multiple factors, such as their growth status, density, and filter-feeding habits. Additionally, *Gracilaria* has the potential to contribute to TSS removal. The adsorption and deposition of particles can effectively decrease the TSS levels in water. The differences in oyster species, experimental conditions, and TSS sources/compositions were the main reasons for the varying research outcomes. In a study on the removal of N and P by Macroalgae, Jiang [62] found that the removal ratios of nitrogen to phosphorus were 2.58 and 2.33, respectively. In this study, when *Gracilaria* was tested for its purification of aquaculture effluent under variations in different environmental factors, it was found that, within 18 days, at a biomass of 2 $kg \cdot m^{-3}$ under a temperature of 20–30 °C and salinity of 10–30‰, *Gracilaria* achieved N to P removal ratios in the aquaculture effluent between 1.93 and 2.87, which is consistent with the findings of previous studies, and the weight gain rate of *Gracilaria* was between 12.90% and 29.89%. However, under the *Gracilaria* + oyster coupling for 48 days, the removal ratio of N to P was 2.41, which is within the range of the previous conditions, and the weight gain rate of *Gracilaria* was 31.01%, slightly lower than the range of changes compared with the former. This indicates that the coupling of the two did not significantly affect the absorption ratios of N and P, but had a certain limitation on *Gracilaria* growth. The reason for this phenomenon may have been that the former were all conducted under laboratory conditions, whereas the latter were conducted under outdoor environmental conditions with a longer experimental period, and were affected by more uncontrollable factors, such as light and temperature differences.

The aquaculture method of Integrated Multi-Trophic Aquaculture (IMTA) systems is acclaimed for its ecological and economic benefits [63]. The *Gracilaria* and oyster combination in this study is a practical implementation of IMTA, which mitigates the environmental degradation of intensive aquaculture by converting waste outputs from one species into resource inputs for another. This combination has exhibited substantial potential in aquaculture effluent purification, consistent with IMTA's core tenets. Future research should concentrate on optimising the spatial configuration and stocking density of *Gracilaria* and oysters to enhance synergistic effects. Moreover, long-term investigations are essential to assess the economic viability and environmental sustainability of this integrated system. IMTA system development must be guided by a comprehensive understanding of ecological interactions and economic trade-offs. It is recommended that future research and development integrate this combined system into a broader IMTA framework, promoting more efficient resource utilisation and diminished environmental impact.

4.5. Deposition Effects of Gracilaria

In practical applications, evaluating the effect of *Gracilaria* on effluent purification based solely on water quality indicators may result in significant errors due to instantaneous changes in the chemical forms of N and P in the water body, as well as the presence of other organisms in the environment. Therefore, it is more scientifically reasonable to evaluate the effect of *Gracilaria* on effluent purification and the resource utilisation of N and P by

measuring their contents in *Gracilaria* biomass at the beginning and end of the experiment, and then calculating the differences in their contents before and after the experiment [64,65]. Wang [66] analysed the N content of Macroalgae, finding values between 1.36% and 5.62%, and that the absorption of N and P nutrients in the water was related to its growth and N storage mechanism. In addition, Fernández-Aláez [67] reported that the absorption rate of nutrients by macroalgae varies due to various factors (such as light and temperature), and the concentration of nutrients such as N and P in the water directly affects the absorption and accumulation by macroalgae. For example, in nutrient-rich waters, macroalgae can absorb more N and P, and their internal N and P content is relatively higher. This was also confirmed in this study. The N and P contents of the surviving *Gracilaria* at the end of the experiment were always higher than those at the beginning of the experiment. The deposition of N and P by *Gracilaria* often reflects its adaptability to the environment and the resource utilisation effect. In this study, the unit net dry weight increment and TN and TP content increments of the *Gracilaria* dry matter at a biomass of 2 kg·m^{-3} were the highest, with values of 19.66, 6.90, and 2.97 mg·g^{-1}, respectively, indicating that the density of *Gracilaria* culture affected the increase in dry weight and N and P deposition by *Gracilaria*, and the appropriate *Gracilaria* culture density was more conducive to effluent resource utilisation. The net dry weight and P content increments of *Gracilaria* at 30 °C were the largest, at 19.74 and 2.85 mg·g^{-1}, respectively, whereas the N content increment at 25 °C was the largest, reaching 3.54 mg·g^{-1}, indicating that a higher-water-temperature environment promoted the deposition of phosphorus and growth of the organism, and the appropriate water temperature promoted the utilisation of nitrogen. The unit net dry weight increment of *Gracilaria* at salinity 30‰ was the highest, which was 19.96 mg·g^{-1}, negative growth occurred at salinity 5‰, and there was no significant difference in the N and P contents of the dry matter between the salinity 10, 20, and 30‰ groups. Thus, the deposition of N and P by *Gracilaria* was not significantly affected by salinity within the range of 10–30‰, and the high salinity conditions were conducive to its own growth and survival, which may have been related to the variety of *Gracilaria* and the contents of various ions in seawater. In practical applications, *Gracilaria* often differs from laboratory conditions due to changes in environmental factors. In this study, the unit net dry weight increment of *Gracilaria* coupled with oysters was −7.77 mg·g^{-1}, which was the opposite of the results of the optimal conditions, while the N and P contents of *Gracilaria* dry matter increased significantly, indicating that the outdoor environment affected the changes in the dry weight contents of organisms, but did not affect its deposition effect on N and P.

In this study, we explored the purification effect of *Gracilaria* and oyster coupling on intensive shrimp farming effluent under optimised conditions (*Gracilaria* biomass 2 kg·m^{-3}, water temperature 25–30 °C, and salinity 15–30‰). The results showed that, for every 1 kg increase in *Gracilaria* dry weight, 22.24–26.76 g and 36.89 g of N, and 10.21–13.48 g and 12.40 g of P were absorbed from the effluent. In 1000 m^3 of effluent, *Gracilaria* + oyster can harvest 63.83 kg of *Gracilaria* dry weight, absorbing 6.38 kg of N and 2.14 kg of P. This indicated better N absorption by *Gracilaria* under coupling. Sun found that Spirulina in fish–algae coupling systems also purified N and P in shrimp farming effluent, with removal rates of 27.7% for TIN and 20.8% for TP [68]. The higher N absorption by *Gracilaria* in this study may have been due to its biological characteristics and differences in experimental conditions compared with Spirulina. In addition, the N released from oyster respiration and metabolism in this study aligns with Flickinger's finding that caged Amazon river prawn and tambaqui increase water N [69]. In summary, *Gracilaria* and oyster coupling showed advantages in N and P removal from intensive shrimp farming effluent, with notably higher N absorption than in other studies. This study offers new purification strategies for shrimp ponds and supports sustainable aquaculture development.

5. Conclusions

(1) The optimal biomass, water temperature, and salinity parameters for *Gracilaria* in the effluent were 2 kg·m^{-3}, 25–30 °C, and 15–30‰, respectively.

(2) The removal of N and P from water did not increase proportionally with an increase in *Gracilaria* biomass. A biomass of 2 kg·m^{-3} showed good purification effects and the best biological deposition of nitrogen and phosphorus.

(3) *Gracilaria* can adapt to water temperatures between 20 and 30 °C. Within this range, higher temperatures are beneficial for the removal and deposition of PO_4^{3-}-P, with 25 °C being optimal for nitrogen deposition and weight gain.

(4) *Gracilaria* is intolerant of environments with salinities below 10‰. The highest weight gain rate was observed at a salinity of 15‰, whereas the highest N and P removal rates were observed at a salinity of 20‰. The depositions of nitrogen and phosphorus showed little difference within the salinity range of 10–30‰.

(5) The coupling of *Gracilaria* and oysters significantly reduced the levels of N and P in water. With 1000 m^3 of effluent, 63.83 kg of *Gracilaria* dry weight could be harvested, absorbing 6.38 kg of N and 2.14 kg of P.

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